October to February, the gonads developed by the treatment without photoperiod change, and the females began to lay eggs earlier than those under natural condition. Their results agree with ours in the importance of rising temperature in spring, but not for the other seasons due to unidentifed reason.

In the wild type medaka, we found that the rising temperature only is responsible for the beginning of spawning season by the same methods as ones in this study using the fish obtained from Ushikunuma pond, Ibaraki Pref. (unpublished data). But Yoshioka [15] indicated that long daylength is indispensable for maturation and spawning in spring in the wild type medaka whose ovary initially contained early yolk-globule state oocytes. This is inconsistent with the present results or with our unpublished data on the wild type medaka. Yoshioka's material was obtained from a pond in Hokkaido (northern Japan), in which changes in photoperiod or temperature is different from those around Tokyo. Difference in environmental conditions during immature period may cause different gonadal response to environmental manipulations in advanced stages. On the other hand, Sawara and Egami [19] reported possible existence of racial difference in photoperiodic response of gonads, which may also be a reason of the inconsistency. To show racial difference clearly, it must be necessary to use material from different localities, which is in the same reproductive stage and has been reared under the same environment.

Ovarian regression was observed in 14°C groups at the end of Experiment 1. The reason of the regression is not clear. But according to Shiraishi *et al.* [26], some females cease spawning after several weeks of daily spawning under constant photoperiod-temperature regimes. The same phenomenon may occur after daily spawning under some experimental conditions employed here. It is also possible that 14°C is too low for maintenance of gonadal maturation.

Observation of yolk globule accumulation in 10 and 12°C groups, and not in 8°C group, suggests two phases of yolk globule accumulation: fast accumulation at above 14°C, slow accumulation at 10 to 14°C. In goldfish Yamazaki [27] obtained similar results by observing slow yolk globule accumulation at 15°C, and fast accumulation at 20 and 25°C in early spring. It is also known that female goldfish can mature at 13 to 14°C, but can not ovulate without temperature rise to 20°C [28]. It will be necessary to ascertain the slow yolk globule accumulation of medaka at 10 and 12°C with longer rearing for understanding relationship between water temperature and maturation or ovulation.

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Fine Structure of Agranular Cells in the Gummy Shark (*Mustelus manazo*) Adenohypophysis

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ABSTRACT—A light and electron microscopic study of the gummy shark (*Mustelus manazo*) adenohypophysis revealed two types of agranular cells (cavity boundary cells and stellate cells) forming a loose network in the distal and ventral lobes. Both types of the cells showed a high nucleocytoplasmic ratio and electron-dense cytoplasm. The cavity boundary cells lining the hypophysial cavities displayed varying numbers of microvilli, occasional cilia and periluminal vesicles with flocculent content. In some of the stellate cells abutting on degenerating endocrine cells, phagocytotic activity was evident. These results suggest that the agranular cells in the shark adenohypophysis may be involved in sustaining the endocrine cells, absorption or transport of substances from the cells and disposition of dysfunctioning endocrine cells.

INTRODUCTION

Agranular (chromophobic) cells have been reported in the adenohypophyses of a wide variety of vertebrates. These cells in lower vertebrates are considered to correspond to folliculo-stellate cells in higher vertebrates and are variously termed as follicle boundary cells, supporting cells, stellate cells, chromophobes and so on [1]. The hypophysis of selachians is characterized by a well-developed cavitary system originating from the recess of Rathke's pouch in the embryo and containing a number of lining agranular cells that are also found dispersed throughout the parenchyma [2–4]. According to Lagios [5], agranular cells are particularly important as supportive cells for primitive fishes bearing large cavities in their hypophysis. Knowles *et al.* [2] and Alluchon-Gérard [3, 4] reported the fine structure of agranular cells in selachian hypophyses, but their function is not clearly established.

The present study was designed to elucidate the fine structure of agranular cells in the gummy shark hypophysis, with the aim of shedding some light on their function(s).

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MATERIALS AND METHODS

Five male gummy sharks (*Mustelus manazo* Bleeker), ranging from 40 to 100 cm in total length, were used in this study. They were caught in a set net installed off the Sado Marine Biological Station of Niigata University, located on the west coast of Sado Island in the Sea of Japan, and reared in an aquarium at the station prior to use.

For transmission electron microscopy, the brains with hypophyses were quickly removed from the skulls immediately after the animals were killed by decapitation under anesthesia with MS-222 and immersed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) containing 1.68% sodium chloride, as recommended by Saito and Tanaka [6]. The hypophyses were cut into small pieces, rinsed in the same buffer, postfixed with 1% osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead solutions and examined with Hitachi H-500 and Jeol 1200 EX electron microscopes.

For scanning electron microscopy, the gland fixed with aldehyde was cut into two halves to

disclose the hypophysial cavities, dehydrated and critical-point dried. After sputter-coating with gold, the specimens were observed with a Hitachi S–500 scanning electron microscope.

Epon semithin sections stained with azan trichrome, aldehyde fuchsin (AF), or periodic acid Schiff's reagent (PAS) were surveyed by light microscopy.

RESULTS

Light microscopy

Light microscopy of the survey sections showed a number of agranular cells scattered over the distal and ventral lobes. These cells were distinguishable from the endocrine cells by their smaller size, irregular outline and darkly stained cytoplasm. Mainly on the basis of their locations, the cells could be classified into two cell types, which coincided with the cavity boundary (pericavity) cells and the stellate cells in *Scyllium* [3, 4]. The former lined the cavitary system (hypophysial cleft in connection with follicular lumina and recess of the ventral lobe), while the latter were interspersed among the endocrine cells. These cells altogether formed a loose network in the parenchyma. The cavities contained varying amounts of AF- and also PAS-positive colloid.

Transmission electron microscopy

The cavity boundary cells were characterized by having a thin electron-dense cytoplasm surrounding an irregularly shaped nucleus (Fig. 1). They were linked to one another by a tripartite junctional complex comprising desmosome, *zonulae occludens* and *adherens* to form the characteristic barrier between the endocrine cells and the cavitary system (Fig. 2). This barrier was incomplete, however, for it was occasionally intercalated with endocrine cells. The cavity boundary cells projected varying numbers of microvilli and one or more cilia into the cavities in which colloid was occasionally demonstrated (Figs. 3 and 4). The colloid was heterogeneous and composed of finely granular material, dense amorphous material, vesicles of various sizes, membranous structures and mitochondria-like bodies, strongly suggesting that it might represent debris derived from disintegrating cells (Fig. 3). Usually, a glycocalyx was spread over the apical surface of the cells. Thin cytoplasmic processes of the cavity boundary cells extended between the endocrine cells and/or abutted on the basement membrane adjacent to the blood vessels. The cytoplasm had relatively few inclusions. In addition to the lack of secretory granules, the presence of abundant free ribosomes, microfilaments and a number of small vacuoles was characteristic of these cells. The vacuoles were scattered throughout the entire cytoplasm, but were mostly concentrated in the subapical region of the cells together with a number of periluminal vesicles having a flocculent content (Fig. 4). Varying numbers of mitochondria and Golgi complexes were present as were electron-dense bodies probably of lysosomal nature, fragments of granular endoplasmic reticulum, multivesicular bodies and myelin figures.

The stellate cells had a star-like configuration, extending their multiple cytoplasmic processes among the endocrine cells (Fig. 5). They were less numerous than the cavity boundary cells and were scattered throughout the parenchyma. Their nucleocytoplasmic ratio was high and no cilia could be seen. However, cytological characteristics of the stellate cells were closely similar to those of the cavity boundary cells. Desmosomes were seldom encountered between the processes of the adjoining stellate cells or between stellate and cavity boundary cells. In the interior of the parenchyma, there were intercellular spaces where the processes of stellate cells and/or cavity boundary cells interdigitated with each other. Usually, these spaces were narrow, but occasionally were dilated and contained amorphous material and vesicular or membranous structures. Noticeably, some stellate cells abutting on disintegrating endocrine cells projected their intricate cytoplasmic processes into the endocrine cells; and present within the processes were phagocytotic vacuoles, some of which were filled with structures clearly recognizable as secretory granules (Fig. 6). The limiting membrane of the granules seemed to be still intact. Small parts of the cytoplasm of the distintegrating endocrine cells appeared to have been engulfed by the processes of the stellate cells. Empty vesicles

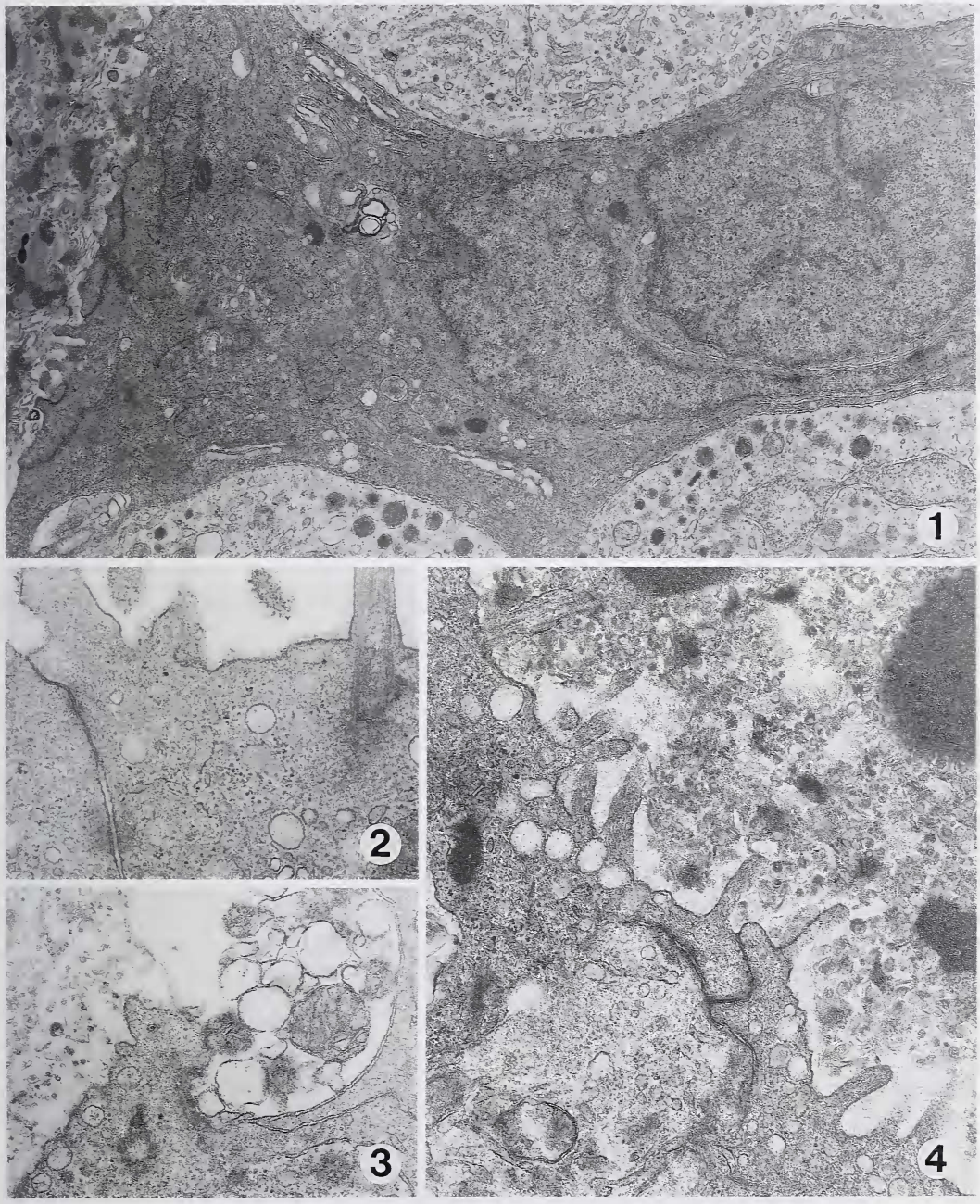

FIG. 1. Cavity boundary cells in the distal lobe of the gummy shark (*Mustelus manazo*) adenohypophysis. Their cytoplasm is electron-dense and contains small vacuoles, a few mitochondria and Golgi bodies. ×10,000.

FIG. 2. A portion of a cavity boundary cell to show the junctional complex between two adjacent cells and a cilium projecting into the lumen. Notice cell surface coated with glycocalyx. ×24,000.

FIG. 3. Apical portion of a cavity boundary cell showing intra-luminal granular materials, vesicles and mitochondria-like bodies. ×18,000.

FIG. 4. Apical portions of cavity boundary cells showing several microvilli, a number of periluminal vesicles with flocculent content. Note the heterogeneous constitution of the intraluminal colloid. ×22,000.

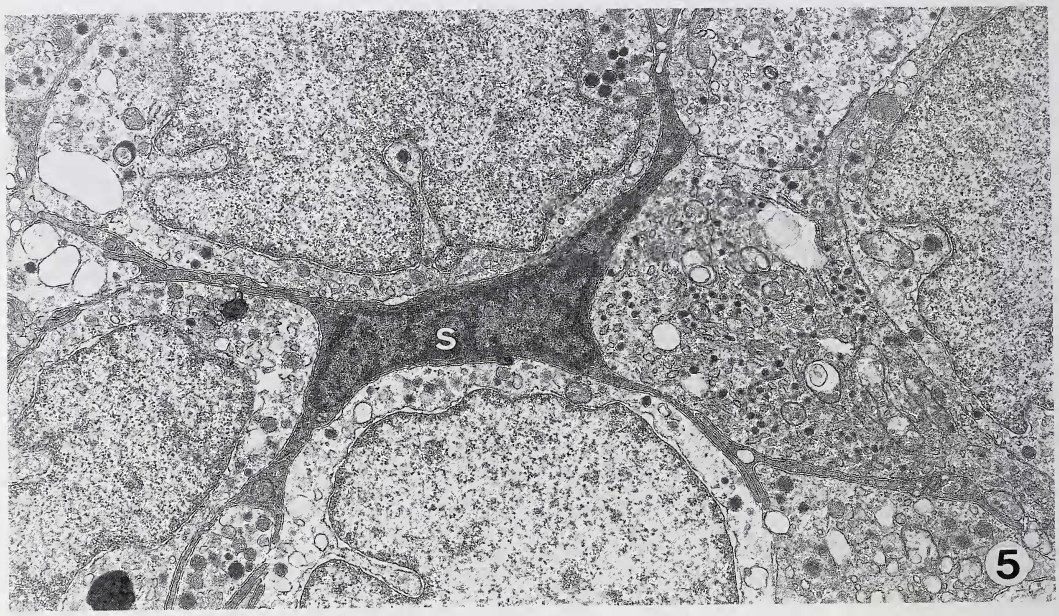

FIG. 5. Stellate cell (S) in the gummy shark adenohypophysis. Note long cytoplasmic processes extending among endocrine cells. ×13,000.

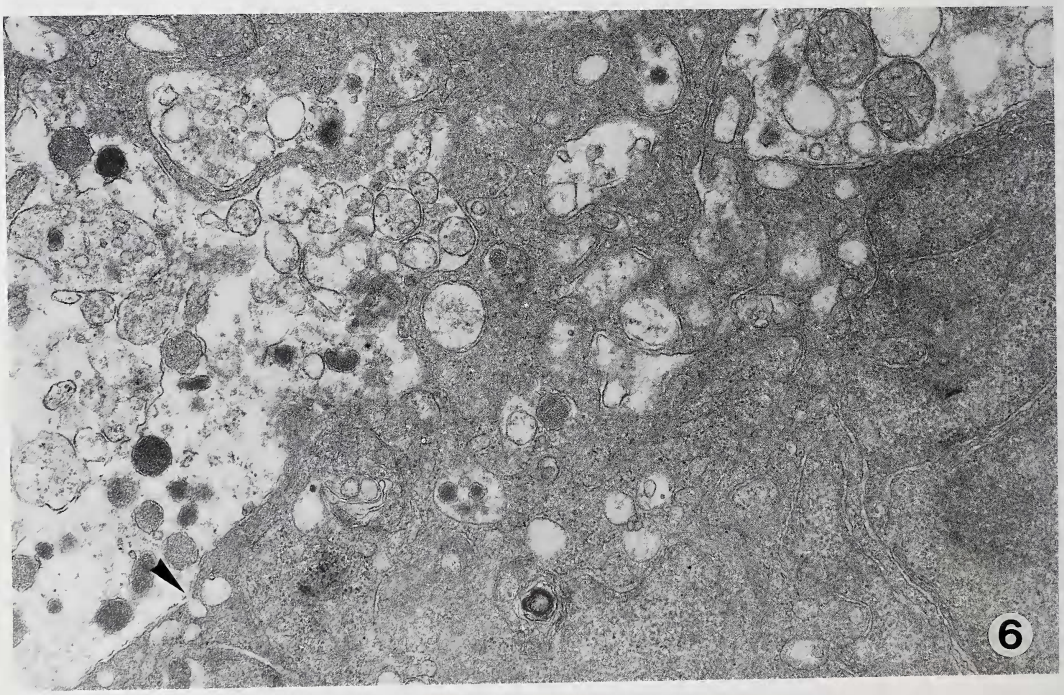

FIG. 6. Portion of a stellate cell showing phagocytotic activity. Note irregular cytoplasmic processes of the stellate cells penetrating into disintegrating endocrine cells. Phagocytotic vacuoles with or without secretory granules, micropinocytotic vesicles (arrowhead) and myelin figure are also visible. ×21,000.